Helminth infections and micronutrients in children

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CHAPTER 7

General discussion

Soil-transmitted helminth infections and micronutrient deficiencies are two major public health issues that occur mostly in the (tropical) developing world and for which children are especially vulnerable. In school-age children, 9.9% of all DALYs lost are attributed to STH infection and micronutrient deficiencies[1]. By combining insights from observational and experimental studies conducted in different countries with different helminth species and endemicity, this thesis addresses the interrelationships between helminth infections and micronutrient status in children. The studies described in this thesis have shown distinct associations between STH infections and micronutrient status in school-age children as well as an increase in helminth infection risk after micronutrient-fortified rice consumption. In this chapter, the results of these studies are integrated, their public health significance discussed and directions for future research are given.

Thesis findings

STH infections and iron

Iron is found in several body compartments and is chaperoned by many different proteins due to the toxicity of its free form[2]. While hemoglobin is often used as an indicator of iron status, hemoglobin concentration can be decreased due to deficiencies in other micronutrients as well[3]. Plasma ferritin concentration is considered to be the best indicator of body iron stores[4]. Our meta-analysis, described in chapter 2, did not support an association between plasma ferritin concentrations and helminth infections. In the Vietnamese study (chapter 3), we also did not find helminth infections to be associated with plasma ferritin, but we did find negative associations of *Trichuris* and hookworm infections with hemoglobin concentration.

Together, these results indicate that while STH infections such as hookworm and *Trichuris* can cause anemia, they may have a smaller effect on iron status than is often assumed[5]. As both hookworm and *Trichuris* burrow into the mucosa, these infections can lead to blood loss. This may diminish stores of hemoglobin, which is predominantly found in blood, but not necessarily iron, which is mostly stored in intracellular ferritin throughout the body. In the long term however, as the process of erythropoiesis is substituting the lost hemoglobin, this may eventually result in a depletion of iron stores. When decreased hemoglobin concentrations cannot be attributed to blood loss or iron deficiency, deficiencies in other micronutrients such as B12 or folate may be the cause[3]. As these micronutrients are also essential to erythropoiesis, their deficiencies might slow down red cell production in iron sufficient persons.

It is likely that the occurrence of an effect on iron status is dependent on helminth infection intensity. A systematic review of hookworm and anemia showed only moderate and heavy hookworm infections to be associated with decreased hemoglobin in school-age children[6]. The hookworm and *Trichuris* infections in the Vietnamese study (chapter 3) were mostly of light intensity. Still, they were associated with lower hemoglobin concentrations and higher anemia prevalence. In the Cambodian study population, hookworm infections (which were also mostly of light intensity) were negatively associated with both hemoglobin and ferritin concentrations (K Khov et al, unpublished results). Interestingly, the meta-analysis on STH infections and iron status in chapter 2 showed an increase in ferritin after deworming. The two studies in the meta-analysis reporting significant rises in ferritin after deworming had been carried out in populations with high endemicity of hookworm infection, where blood loss may have been so severe that the infection led to decreased iron stores[7, 8].

In addition to the importance of infection intensity, helminth species also appears to be a determinant of iron status. Although hookworm species are usually not differentiated in epidemiological studies, *Ancylostoma* infection has been shown to result in more blood loss and lower ferritin concentrations than *Necator*[8]. This difference could partly explain heterogeneity between studies assessing the effect of hookworm infection on anemia or iron deficiency in the literature.

It should be noted that incomplete adjustment for inflammation in chapters 2 and 3 may have occluded a rise in ferritin caused by inflammation during STH infection. To withhold this scarcely bioavailable nutrient from pathogens, iron is sequestered into macrophages during acute phase immune responses[9]. This phenomenon can make measurements of iron status by plasma ferritin unreliable. Therefore, it is recommended to use markers of the acute phase response (C-reactive protein and alpha-1-acid glycoprotein) to adjust for the rise in ferritin that occurs during inflammation[10]. Although we did not find STH infections to be associated with acute phase responses in Cuba and Cambodia (chapter 5), it cannot be ruled out that concurrent infections causing acute phase responses may have diminished the validity of the plasma ferritin measurements.

Figure 7.1 gives a schematic representation of the findings of this thesis on STH infections and indicators of iron status. In short, an association between STH infections and iron status as measured by plasma ferritin was not supported by our studies (chapter 2 and 3). However, the results do support an association between STH infection and hemoglobin concentration and/or anemia (chapter 3). This may either represent a direct effect of blood loss, or an indirect effect via deficiencies of other micronutrients such as folate or vitamin B12. The incomplete adjustment for inflammation impairs the validity of our results on ferritin. While STH infections did not seem to cause a rise in either CRP or AGP (chapter 5), other (co-)infections or inflammatory conditions could have confounded our plasma ferritin measurements.

STH infections and vitamin A

Our meta-analysis of observational studies on STH infections and vitamin A status revealed a significant negative association (chapter 2). While species-specific subgroup analyses were not feasible for the meta-analysis, the findings in chapter 3 confirm that a decrease in plasma retinol concentrations is characteristic for *Ascaris* infections. The localization of *Ascaris* may explain this phenomenon: the large adult worms can obstruct the jejunum or interfere with proper mixing of the food with bile salts needed for the absorption of fats and fat-soluble vitamins such as vitamin A[11]. This is less likely for infections with hookworm, which is a much smaller organism, or for *Trichuris*, of which

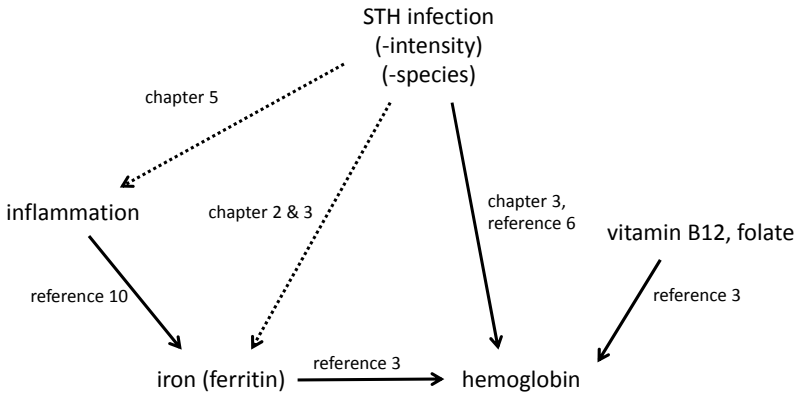

Figure 7.1: Associations (depicted by arrows) between soil-transmitted helminth infection, iron status and inflammation described in this thesis. Dashed arrows represent associations that were not supported by the studies described in this thesis.

the adult worms are localized in the colon. The question remains whether *Ascaris* infection also has consequences for less-studied vitamins that are also fat-soluble (vitamins D, E, and K) plus fat itself. A study of primary biliary cirrhosis, a disease wherein bile salt delivery to the intestinal lumen is also impaired, found adult patients to have few deficiencies in fat-soluble vitamins other than vitamin A[12].

Our meta-analysis in chapter 2 did not show an increase in plasma retinol after deworming. It is possible that study follow-up periods were insufficient to measure vitamin A repletion. Alternatively, quick reinfection may hamper repletion of vitamin A. Another possibility is that retinol is not sensitive enough as a marker to measure increases in vitamin A stores. When liver vitamin A stores suffice, plasma retinol concentrations are kept fairly constant. Only in the context of (sub)clinical deficiency does plasma retinol reflect individual vitamin A status[11, 13]. Therefore, rises in plasma retinol would only be expected when infected children are vitamin A deficient at baseline and when this deficiency is resolved (shortly) after deworming. Again, insufficient adjustment for inflammation is an important limitation of our results, because retinol decreases in the blood during the acute phase response[10]. Despite the fact that our findings in chapter 5 contradict acute phase responses during STH infections, we should ideally have adjusted for these.

STH infections and zinc

Zinc deficiency is recognized as an important cause of stunting. If STH infections contribute to the risk of zinc deficiency and/or its effect on growth, this is an important finding for public health. The literature search for our review of chapter 2 showed a paucity of published studies on plasma zinc concentrations and helminth infections. The three studies for which we were able to extract data found either no association or a slight negative association between helminth infection and plasma zinc concentration[14-16]. In chapter 4, we examined associations between STH infections, height for age and (hair or plasma) zinc levels in Cuban and Cambodian children. We found associations between zinc and height for age in both Cuba and Cambodia. In the Cambodian study population, STH infected children had on average lower plasma zinc concentrations. In Cuba, hair zinc was not associated with STH infection, and both STH infection and plasma zinc concentration appeared to be independent determinants of height for age. In the Vietnamese study, described in chapter 3, plasma zinc tended to be higher in STH infected children. This discrepancy might be attributable to the lack of adjustment for AGP concentrations in the Vietnamese study, while the Cambodian plasma zinc analysis included both CRP and AGP concentrations as covariates[10].

Possible mechanisms through which zinc could be related to STH infection in humans have not been studied so far. Zinc is normally excreted and re-absorbed in the intestine, and diarrhea has been shown to increase loss of zinc in feces. Possibly, STH infections impair absorption or re-absorption of zinc. Conversely, zinc deficiency is known to increase susceptibility to infections. In several animal studies, zinc deficiency has been shown to promote helminth survival [17, 18].

The lack of a good biomarker to measure true zinc status is a major hurdle in studying determinants of zinc deficiency. In addition, while it is known that plasma zinc, like ferritin and retinol, responds to inflammation, plasma zinc correction factors for inflammation in children and adolescents are not yet available[10]. Moreover, zinc is suggested to be a type II nutrient, meaning that the body responds to dietary deficiency by stopping growth and thereby keeping tissue concentrations relatively stable[19]. Therefore, zinc deficiency is very difficult to measure. The only way to diagnose zinc deficiency would be to supplement zinc and monitor growth: if a child shows enhanced growth after zinc supplementation, he or she was probably zinc deficient. However, even then a co-existing growth-limiting (type II) deficiency (such as magnesium or potassium) could still inhibit growth, thereby occluding the effect of the supplemented zinc[19]. The difficulties in assessing zinc status have hindered estimations of zinc deficiency burden and its health effects. Fortunately, zinc is gaining attention as an important nutrient and as an opportunity for public health strategies to improve child health and development[20].

STH infections and iodine

We were able to find one study addressing iodine and helminth infections for our review in chapter 2. This study reported increased effectiveness of iodized oil administration when it was combined with deworming[21]. However, this observation may be related more to the absorption of oil which may have been reduced in *Ascaris* infection than that of iodine per se. In our Vietnamese study described in chapter 3, we did not find STH infection and urinary iodine excretion to be associated. No other reports exist in the published literature about an association between iodine and helminth infection.

STH infections and inflammation

Despite its name, the acute phase response can persist over long periods, especially in the increasingly common context of chronic diseases. The presence of such a response is relatively easy to measure by acute phase proteins in plasma (such as C-reactive protein and alpha-1 acid glycoprotein). In chapter 5, we found that Cuban and Cambodian STH infected children did not present with more plasma acute phase proteins than their uninfected peers. In addition, the presence of elevated fecal calprotectin, a marker for local intestinal inflammation[22], did not differ between STH infected and uninfected children in both study populations. The lack of inflammatory responses in STH infected children was not surprising, considering that helminths are widely known for their potent anti-inflammatory capacities[23]. Clinical trials are even exploring the potential of helminths as anti-inflammatory therapeutics in inflammatory bowel disease[24]. Nevertheless, our results can provide new insights for the field of nutrition and infectious disease. Firstly, the lack of systemic inflammation observed in STH infections excludes these infections as a factor causing changes in plasma micronutrient concentration via the induction of acute phase responses and thus confounding micronutrient measurements. Second, the lack of association between STH infection and fecal calprotectin does not support mucosal damage as a mechanism by which helminth infections could decrease nutrient absorption. On the other hand, mucosal damage causing inflammation characterized by eosinophilia is still a possibility, as this might not result in increases in fecal calprotectin. In that case, calprotectin would thus be an inappropriate biomarker for helminth-induced intestinal inflammation.

(Multi-)micronutrient administration and STH infections

The meta-analysis presented in chapter 2 showed a trend towards lower helminth (re)infection after multi-micronutrient supplementation. This is in contrast with our findings in chapter 6, where we found an increased risk of hookworm infection after consumption of multi-micronutrient fortified rice in school meals. However, when we perform the meta-analysis including only hookworm as outcome and when we add the findings described in chapter 6, the pooled effect estimate supports an increase in infection risk after multi-micronutrient administration (Figure 6.2).

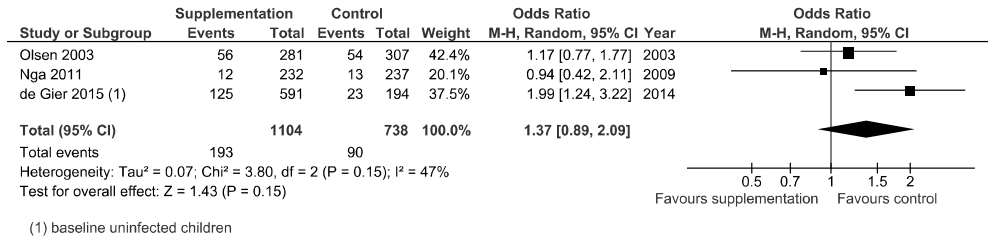

Figure 6.2. Random-effects meta-analysis of randomized controlled trials on effects of multi-micronutrient supplementation or fortification on hookworm infection.

The mechanism by which hookworm infection risk is increased by fortified rice is unclear. Since pathogens such as hookworm need essential micronutrients themselves, the nutrients in the rice may have contributed to parasite survival. This 'feeding the parasite'-theory is in agreement with the concept of nutritional immunity, which is mostly described in the context of iron metabolism. Indeed, iron deficiency has been shown to be protective against *P. falciparum* malaria and other infections, underlining the pathogen's need for this micronutrient[25, 26]. Feeding the parasite seems especially plausible for enteric pathogens. On the other hand, hookworm is not known to feed on luminal contents and would therefore not directly profit from micronutrient-enriched food. Nevertheless, high doses of a nutrient that is strongly coveted by the parasite may still indirectly facilitate infection, assuming that high concentrations in the gut lumen result in increased enteric absorption and thus increased micronutrient content of mucosal tissue and blood.

Interrelationships between nutrition and helminth infection may also be mediated by the gut microbiota. Possibly, multi-micronutrient fortified rice had an effect on small intestine microbiota favoring hookworm establishment. It is known that diet can affect microbiota composition[27]. In turn, certain microbiota compositions may favor or hinder helminth colonization[28]. Remarkably, in a murine infection model, *Trichuris muris* eggs have been

shown to require bacterial cell contact for hatching[29]. Moreover, the intestinal nematode *Nematospiroides dubius* is less able to establish and maintain infection in germ-free mice than in wild-type mice[30]. Conversely, the presence of helminths in the intestine may influence microbiota composition as well[28]. Not much is known about the relationship between microbiota and helminths in humans. Two recent studies in humans reported no significant associations of either hookworm or *Trichuris trichiura* infection with fecal microbiota composition[31, 32]. However, another recent study did find STH infection to be associated with increased microbial diversity[33]. Preliminary analysis of data from the Cambodian trial described in chapter 6 did not show hookworm infection to be associated with changes in fecal microbiota. Perhaps, fecal microbiota compositions fail to reflect local changes in those parts of the intestinal tract that are inhabited by the helminths[31]. For example, infections specific to the small intestine such as hookworm may indeed affect the local microbiota composition, but this specific effect may not be measurable anymore once the fecal matter has passed through the heavily colonized large intestine.

Lastly, multi-micronutrient administration can elicit nutrient interactions. As minerals such as iron and zinc compete for absorption, high doses of one may preclude absorption of the other, thereby perhaps inducing one deficiency by repleting the other. Indeed, iron and zinc have been reported to influence each other's absorption or health effects[34]. Consequently, the induced or exacerbated deficiency may impair immune responses to infection. A recent meta-analysis showed that for reducing diarrhea and improving height, zinc supplementation alone is more effective than zinc co-administered with iron[35].

Public health implications

A solid evidence-base is essential to avoid wasting scarce resources for child health advancement on ineffective interventions. The global health community was obliged to re-evaluate its view on deworming and micronutrient administration when in 2013, the results of the 'DEVTA trial' were published. This study, following one million Indian children, observed no effect of either albendazole treatment or vitamin A supplementation on mortality[36]. Moreover, albendazole administration was not associated with weight, height or hemoglobin improvements either[36]. While the DEVTA study design was highly criticized, it has highlighted a need for rethinking global health programs. The current practices for improving child health and nutritional status such as deworming and micronutrient (co-)administration are arguably not justified by sufficient evidence[37].

Deworming and micronutrient administration programs

The studies described in chapters 2, 3 and 4 have shown that associations exist between helminth infections and micronutrient status. However, a large evidence gap remains concerning the effects of deworming on micronutrient status. While observational studies continue to report strong associations between helminth infections and height, weight and/or hemoglobin levels, a Cochrane review could not confirm positive effects on child growth after deworming[38]. This may indicate that no association exists between helminth infection and nutritional status, however deworming trials measuring changes in nutritional status are hampered by rapid reinfection and short follow-up time[39]. Approximately 30% of children become reinfected within three months after treatment for *Ascaris, Trichuris* and hookworm through the sustained presence of STH eggs and larvae in the environment[40]. Replenishment of micronutrients and catch-up growth would likely require longer periods free of helminth infection. Long-term follow-up of successfully dewormed children, while preventing reinfection, is needed in order to assess the true effect that removal of helminth infection has on nutritional status. Sustainable interventions against helminth (re)infections may have to be sought in safe water supply, adequate sanitation and hygiene (WASH)[41]. This is also expected to exert a positive impact on many other infections and child nutritional status. Indeed, previous research has shown that WASH can positively affect child growth and can impact access to and absorption of micronutrients[42].

While the WHO, UNICEF and the Copenhagen consensus advocate combining deworming with micronutrient administration in helminth-endemic areas, our results show that these recommendations should be used with caution (chapter 6)[9, 43]. Micronutrient administration may impair child health in helminth endemic areas (chapter 6). Deworming programs will not eliminate this risk, as cure rates are not 100% and reinfection is the rule rather than the exception, especially in high transmission areas[40, 44]. The merits of micronutrient repletion should be weighed carefully against its possible risks. Unfortunately, as our results in chapter 6 have shown, the risks and benefits may differ greatly even per school area. This focal character of infection risk may complicate the identification of optimal health and nutrition interventions for populations.

There is insufficient evidence on possible effects of micronutrient supplementation on the efficacy of deworming programs or vice versa[5]. Nevertheless, co-administering micronutrients with anthelminthics remains an opportunity to benefit children who are at risk for both micronutrient deficiencies and helminth infections[5]. The associations found in this thesis support this co-occurrence. However, safety is of utmost importance when combining micronutrient administration with anthelminthics, to avoid an even further increase in disease risk for these underprivileged children.

Helminths and the nutrition transition

When considering nutritional matters in low- and middle income countries, it is essential to do this in the context of the rapidly changing food landscape. The transition from a situation with predominant undernutrition toward one of high availability of low-quality food, resulting in a dramatic increase in chronic metabolic diseases, is now a reality in many tropical countries[45]. In the classic description of the epidemiological transition by Omran in 1971, this shift coincides with a drastic decrease in infection pressure. However, this does not always hold true in the helminth-endemic tropics[46, 47]. While STH prevalence has decreased considerably in Asia between 1990 and 2010, prevalence has remained quite stable in Latin America and Sub-Saharan Africa[48]. During these decades, a new reality has emerged wherein diseases such as obesity and type 2 diabetes co-occur with helminth infections.

The studies described in this thesis have found distinct associations between helminth infections and micronutrient status in children to exist in at least three countries at different places on the nutrition-transition-spectrum. While Cambodia is still a low-income country with predominant undernutrition and a stable, low prevalence of obesity, its neighbor Vietnam has seen a dramatic increase in obesity and metabolic disorders in the last two decades[49-51]. Cuba has also made the transition towards high prevalence of diabetes and obesity, although Cuba is quite unique regarding its food landscape which is less diverse than that of open-market economies[52, 53].

The shift in food landscape during nutrition transition may well exacerbate micronutrient deficiencies because of the low-quality foods that increasingly dominate the diet. Indeed, in a recent study in Vietnam, overweight was often accompanied by micronutrient deficiency in the same individuals, irrespective of socioeconomic status[54]. Also, micronutrient deficiencies may contribute to an increasing prevalence of the so-called metabolic syndrome, a condition that characterizes the nutrition transition[55-57].

Early life malnutrition is associated with cardiovascular risk in adulthood[58]. Therefore, negative effects of helminth infection on nutrition in children or pregnant or nursing mothers may contribute to cardiovascular disease later in life. The nutrition transition is giving rise to 'dual burden households', i.e. households with underweight and overweight persons[59]. This dual burden household phenomenon has been found to be associated with intestinal parasite infection in Venezuela, suggesting a role for parasite infection within this complex phenomenon[60].

In addition, our results have highlighted the possibility that the anti-inflammatory effects of helminth infections may also modify the risk of metabolic syndrome. Metabolic syndrome is accompanied by C-reactive protein increases, which are independent strong predictors of cardiovascular events[61]. The trend towards lower CRP described in chapter 5, along with reports from others, hints towards helminth infections attenuating the risk for cardiovascular diseases, although further studies are needed to test this hypothesis.

Conclusions and further research directions

This thesis describes distinct associations between helminth infections and micronutrient status in school-age children. Iron, vitamin A and zinc were found to be associated with helminth infections; these associations appear to be dependent on helminth species, intensity of infection and other population characteristics. Soil-transmitted helminth infections were not associated with acute phase inflammation markers or with local intestinal inflammation. We found an increase in hookworm infection risk after consumption of micronutrient-fortified rice in a randomized, placebo-controlled trial. Together, our results show that helminth infections and micronutrient status, two major public health concerns worldwide, are interrelated.

Further studies are needed to shed more light on mechanisms by which multi-micronutrient fortified food consumption might increase hookworm infection risk, and which population or location characteristics can modify this effect. Insight into such mechanisms will help evaluate the safety of forms and doses of micronutrients in helminth-endemic areas. While research has focused mainly on iron, vitamin A and, to a lesser extent zinc, many other essential micronutrients are thus far understudied. The B vitamins, folate, calcium and many other micronutrients are often part of multi-micronutrient interventions but have hardly been studied in association with helminth infections.

Unraveling the forces at play in the human intestinal ecosystem will contribute to optimizing nutritional and anthelminthic interventions. The intestinal microbiota may be a potent modifying factor in the relationship between intestinal infection and nutrition. Minimally invasive methods (as opposed to endoscopy) are needed for epidemiological studies to safely obtain samples from specific intestinal sites in order to reliably study relationships between helminth infection, nutrition and microbiota in humans. As helminth infections seldom occur in isolation, co-infections with protozoa, bacteria and viruses also need to be considered in future studies addressing helminth-nutrition associations.

In addition, it is essential to gain insight into the helminth-micronutrient interplay within the context of the nutrition transition. The mechanisms involved in the interrelationships between undernutrition, overweight/obesity and parasite infection within populations and even households will likely be able to affect micronutrient status in children as well. Interdisciplinary or mixed-method studies are needed to study the complex interactions between biological and socioeconomic risk factors of dual burden households and helminth infections.

Policies on micronutrient delivery and deworming need to be based on solid evidence from studies conducted in relevant populations. Future research should focus on redesigning, optimizing and combining interventions to reduce helminth infections and micronutrient deficiencies. As these diseases are interrelated, addressing either one of them separately would be less effective as well as a missed opportunity to increase child health in the developing world. Considering the high reinfection rates despite current mass deworming strategies, efforts to reduce helminth burden should be directed more toward improving sanitation and hygiene. Such improvements are likely to not only reduce helminth infections but also many other protozoan and bacterial infections impairing intestinal function, and will thereby greatly benefit child health and nutritional status.

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